Conversion of chlorinated propanes by Methylosinus trichosporium OB3b expressing soluble methane monooxygenase

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Conversion of chlorinated propanes by *Methylosinus trichosporium* OB3b expressing soluble methane monooxygenase

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Abstract Chlorinated propanes are important pollutants that may show persistent behaviour in the environment. The biotransformation of 1-chloropropane, 1,2-dichloropropane, 1,3-dichloropropane and 1,2,3-trichloropropane was studied using resting cell suspensions of *Methylosinus trichosporium* OB3b expressing soluble methane monooxygenase. The transformation followed first-order kinetics. The rate constants were in the order 1-chloropropane > 1,3-dichloropropane > 1,2-dichloropropane > 1,2,3-trichloropropane, and varied from 0.07 to 1.03 ml min^{-1} mg of $cells^{-1}$ for 1,2,3-trichloropropane and 1-chloropropane respectively. Turnover-dependent inactivation occurred for all of the chloropropanes tested. The inactivation constants were lower for 1-chloropropane and 1,2-dichloropropane than for 1,2,3-trichloropropane and 1,3-dichloropropane. Not all the chloride was released during cometabolic transformation of the chlorinated propanes and production of monochlorinated- and dichlorinated propanols was found by gas chromatography. The reaction pathway of 1,2,3-trichloropropane conversion was studied by mass spectrometric analysis of products formed in $^{2}H_2O$, which indicated that 1,2,3-trichloropropane was initially oxidized to 2,3-dichloropropionaldehyde and 1,3-dichloroacetone, depending on whether oxygen insertion occurred on the C-3 or C-2 carbon of 1,2,3,-trichloropropane, followed by reduction to the corresponding propanols. The results show that chloropropanes are susceptible to cometabolic oxidation by methanotrophs, but that the transformation kinetics is worse than with cometabolic conversion of trichloroethylene.

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Introduction

Industrial spillage and agricultural usage are the main causes of environmental pollution with chlorinated propanes. These compounds are used as solvents, soil fumigants and intermediates in chemical synthesis. During the synthesis of epichlorohydrin, 1,2,3-trichloropropane is formed as a by-product. Commercial preparations of the nematocide 1,3-dichloropropene, which is used on potato crops, often contain 1,2-dichloropropane. Chloropropanes are suspected carcinogens that frequently occur as contaminants of groundwater and are poorly degraded in the environment and in biological treatment systems. Microbial growth on chlorinated propanes has only been well documented for 1-chloropropane and 1,3-dichloropropane (Janssen et al. 1985), but not for 1,2-dichloropropane and 1,2,3-trichloropropane. However, cometabolic degradation has been demonstrated for 1-chloropropane and 1,2-dichloropropane (Shimoda et al. 1993; Oldenhuis et al. 1989). Recently anaerobic conversion has been described for 1,2-dichloropropane (Löffler et al. 1997). More highly chlorinated compounds, such as 1,2,3-trichloropropane, are in general very resistant to biodegradation, and this contributes to their persistence in the environment.

In the past decade research has shown that methanotrophs can cometabolically degrade halogenated aliphatic hydrocarbons as a result of the broad substrate range of their methane monooxygenase (Oldenhuis et al. 1989). The methanotroph *Methylosinus trichosporium* OB3b expresses under copper limitation a soluble methane monooxygenase. This enzyme catalyses a wide range of oxidation reactions, including the hydroxylation of alkanes, the epoxidation of alkenes, and the oxidation of ethers, halogenated alkanes and alkenes, and aromatic compounds (Colby et al. 1977; Oldenhuis et al. 1989; Shimoda et al. 1993; Sullivan and Chase 1996). The soluble methane monooxygenase of this organism converts halogenated aliphatics via different

reaction mechanisms, depending on the compound. Mono- and polyhalomethanes were found to be converted by insertion of oxygen into the carbon-hydrogen bond (Bartnicki and Castro 1994). A sequential oxidative and reductive pathway has been suggested for the conversion of vinyl chloride, leading, via chloroethylene oxide, to glycolic acid, ethylene glycol and chloroacetic acid (Castro et al. 1992). Direct insertion of oxygen into the carbon-halogen bond has been proposed for 1,2-dichloroethane, chloroacetic acid and chloroacetamide (Riebeth et al. 1992; Castro et al. 1996). Similar reaction mechanisms have been proposed for cytochrome *P*-450 (Castro et al. 1985; Castro and Belser 1990).

Little is known about the possibility of degrading chlorinated propanes by methanotrophs and the mechanism by which they are converted. The purpose of this work was to determine the degradation kinetics of the cometabolic conversion of chlorinated propanes by *M. trichosporium* OB3b. The transformation products were analysed and the reaction pathway of 1,2,3-trichloropropane was investigated in more detail.

Materials and methods

Organism and growth conditions

Methylosinus trichosporium OB3b (NCIMB 11131) was obtained from the National Collection of Industrial and Marine Bacteria, Aberdeen, UK. The organism was grown continuously in a 3-l fermentor as described by van Hylckama Vlieg et al. (1996).

Preparation of suspensions of *M. trichosporium* OB3b

Cells grown in a fermentor were harvested by centrifugation ($6000 \times g$ for 5 min at 4 °C) and suspended in mineral medium supplemented with 20 mM phosphate buffer (pH 6.9) and 20 mM sodium formate. The mineral medium was the same as that described by Janssen et al. (1985). Suspensions prepared in this way were used for degradation experiments in batch cultures. These were performed in 25-ml incubations in shake flasks (30 °C) at a substrate concentration of 0.5 mM. Samples were taken at different times and analysed by gas chromatography.

For the deuterium oxide experiments, mineral medium supplemented with 20 mM phosphate buffer and 20 mM sodium formate was freeze-dried, and dissolved in deuterium oxide. The cells were resuspended in this buffer and incubated for 10 min to allow exchange of water. After centrifugation the cells were resuspended in the same buffer and incubated at 30 °C with 0.5 mM 1,2,3-trichloropropane for 2 h. Products were extracted with diethyl ether and concentrated 200-fold by evaporation of the solvent prior to GC-MS analysis.

Treatment with cyclopropane, which can be used to inhibit methanol dehydrogenase selectively, was performed by incubating the cell suspension with 1 mM cyclopropane for 3–4 min at 30 °C. The excess cyclopropane was removed by air bubbling.

Degradation kinetics

Cometabolic degradation of 1-chloropropane, 1,2-dichloropropane, 1,3-dichloropropane and 1,2,3-trichloropropane by the soluble monooxygenase was determined by on-line analysis of their concentrations in the headspace (van Hylckama Vlieg et al. 1996). The system consisted of a 120-ml double-walled glass incubation vessel that was temperature-controlled at 30 °C. Gas was continuously withdrawn from the headspace and injected back into the magnetically stirred liquid phase with a flow rate of approximately 200 ml min^{-1}. At intervals of 1 min, the content of the sample loop (35 µl) was injected into a gas chromatograph. The instrument (Chrompack type CP9001) was equipped with a CPSil-5-CB column (Chrompack; length 25 m, inner diameter 0.53 mm, film thickness 5 µm), and a flame ionization detector. Helium was used as the carrier gas (175 kPa), and the column temperature was 114 °C.

To determine the degradation kinetics with 1-chloropropane, 1,2-dichloropropane and 1,3-dichloropropane, 25 ml cell suspension [0.4 mg cells (dry weight) ml^{-1}] was taken directly from the fermentor. For degradation of 1,2,3-trichloropropane the cells were concentrated fourfold. Formate and phosphate buffer (pH 6.9) were added to a final concentration of 20 mM each. The cell suspension was incubated for 5 min to allow generation of enough reducing power to obtain a maximal initial degradation rate. Assays were then started by adding halogenated substrate from a stock solution in water to a final concentration of 50 µM in the liquid phase.

Analytical methods

Polar halogenated compounds produced during the degradation experiments were analysed on a Chrompack 438S gas chromatograph equipped with a flame ionization detector and a CPWax-52-CB column (Chrompack; length 25 m, inner diameter 0.25 mm). The carrier gas was nitrogen (50 kPa), and the temperature programme was 3 min at a constant temperature of 45 °C followed by an increase to 200 °C at 10 °C min^{-1}. Samples (4.5 ml) were extracted with 1.5 ml diethyl ether containing 0.05 mM 1-bromohexane as the internal standard. The upper layer was analysed by split injection of 1-µl samples in the gas chromatograph. The identity of the degradation products was confirmed by comparison of the retention time with those of authentic standards.

GC-MS analysis was performed on a HP 5890 gas chromatograph with a HP5 capillary column (length 25 m, inner diameter 0.25 mm, film thickness 0.25 µm), connected to a flame ionization detector and a type-5971 mass-selective detector. Helium was used as a carrier gas (0.9 ml min^{-1}), and the temperature programme was 3 min at a constant temperature of 45 °C followed by an increase to 200 °C at 10 °C min^{-1}.

Halide levels were determined by the colorimetric method of Bergmann and Sanik (1957).

Chemicals

Halogenated compounds were obtained from Janssen Chimica, Beerse (Belgium), or from Merck, Darmstadt (Germany). ^2H_2O (99.8% v/v) was purchased from Isotec Inc., Miamisburg, Ohio (USA). Cyclopropane was obtained from Aldrich Chemie, Bornem (Belgium).

Results

Degradation kinetics of chlorinated propanes

Cell suspensions of *M. trichosporium* OB3b expressing the soluble methane monooxygenase were used for degradation experiments. The cometabolic conversion of four different chlorinated propanes was followed by on-line gas chromatography. At the substrate concentration used (50 µM), the transformation of all compounds followed first-order kinetics. Substrate-depletion curves for 1,2-dichloropropane are shown in Fig. 1A. The first-order rate constants (k_1), obtained after the first

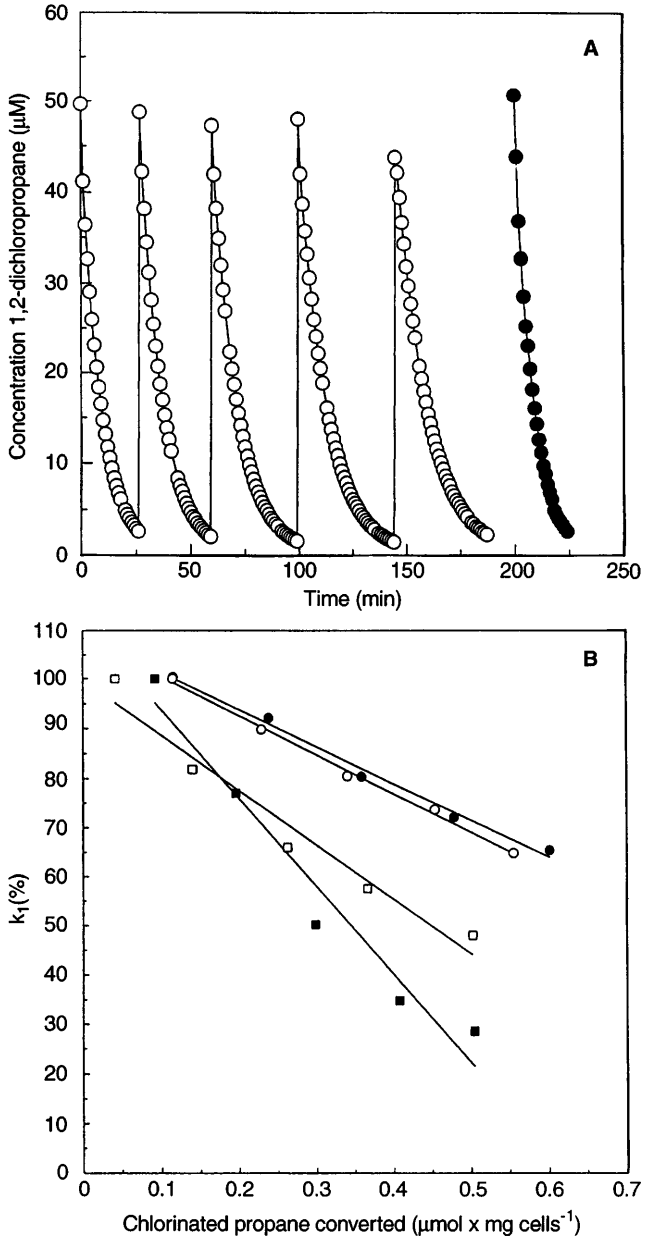

Fig. 1A, B Effect of repeated additions of chloropropanes on the degradation rate by *Methylosinus trichosporium* OB3b cells. **A** Repeated degradation of 1,2-dichloropropane. As a control, degradation was also measured with cells to which 1,2-dichloropropane had been added for the first time after 200 min (●). **B** Effect of the amount of chlorinated propane converted on the first-order rate constant expressed as a percentage of the initial rate constant. (●) 1-Chloropropane, (○) 1,2-dichloropropane, (■) 1,3-dichloropropane, (□) 1,2,3-trichloropropane

addition of the substrate, decreased in the order of 1-chloropropane, 1,3-dichloropropane, 1,2- dichloropropane and 1,2,3-trichloropropane (Table 1).

Cometabolic conversion of chlorinated hydrocarbons may result in turnover-dependent inactivation of the soluble methane monooxygenase (Fox et al. 1990; Oldenhuis et al. 1991). The inactivation caused by conversion of chlorinated propanes was tested by monitoring substrate-depletion rates after repeated additions of these compounds to a concentration of 50 μM. The repeated addition of 1,2-dichloropropane caused a lower degradation rate after each subsequent addition (Fig. 1A, B). No decrease in the degradation rate occurred with a control culture to which the halogenated substrate had been added for the first time after 200 min. Similar depletion curves were determined for the other chloropropanes. The relative first-order rate constants were plotted against the amount of chloropropane converted (Fig. 1B), and from this the inactivation constants were determined (Table 1). Similar inactivation constants were observed for 1-chloropropane and 1,2-dichloropropane. Both 1,2,3-trichloropropane and 1,3-dichloropropane showed higher inactivation constants, and the degree of inactivation per amount of substrate converted decreased when the cells became less active. This may be due to inactivation being caused by accumulation of unstable reactive products, which would reach lower levels if their rate of formation decreased after each subsequent pulse. All chloropropanes were more toxic than trichloroethylene, for which conversion-mediated inactivation is well established (Oldenhuis et al. 1991).

Analysis of products of chlorinated propane transformation

Various intermediates formed during conversion of chlorinated propanes by *M. trichosporium* OB3b were identified by GC analysis of ether extracts of the incubation mixtures. Chlorinated propanols were found as degradation products of 1-chloropropane, 1,2-dichloropropane and 1,2,3-trichloropropane (Table 2).

During conversion of 1-chloropropane, 67% of the organic chlorine was liberated and 1-chloro-2-propanol was formed in low amounts. In order to determine the primary products of chloropropane transformation, we tested whether additional alcohols were produced if further degradation was inhibited with cyclopropane. Cyclopropane and cyclopropane-derived compounds are irreversible covalent inhibitors of the methanol dehydrogenase, which oxidizes several primary alcohols (Dijkstra et al. 1984; Shimoda and Okura 1991; Shimoda et al., 1991; Yamada et al. 1992). For these experiments, 20 ml cells [0.3 mg cells (dry weight) ml^{-1}] freshly taken from a fermentor were incubated at 30 °C for 2 h with 1-chloropropane or 3-chloro-1-propanol with or without pretreatment with cyclopropane (1 mM). During 1-chloropropane conversion low amounts of 1-chloro-2-propanol were formed both with cyclopropane-treated cells and with untreated cells. Incubation of cyclopropane-treated cells with 1-chloropropane (0.5 mM) yielded mainly 3-chloro-1-propanol (0.3 mM), and 1-chloro-2-propanol (0.024 mM). With 3-chloro-1-propanol (0.5 mM) complete degradation was observed only for non-treated cells and up to 85% of the organic chlorine was released as inorganic chloride. With

Table 1 First-order rate constants and inactivation constants for the degradation of chlorinated propanes by *Methylosinus trichosporium* OB3b cells expressing soluble methane monooxygenases

Substrate	k_1 (ml mg cells^{-1} min^{-1})	C_1 (mg cells inactivated μmol substrate converted^{-1})
1-Chloropropane	1.03	0.68
1,2-Dichloropropane	0.36	0.67
1,3-Dichloropropane	0.58	1.60
1,2,3-Trichloropropane	0.07	1.11
Trichloroethene[a]	3.10	0.30

[a] van Hylckama Vlieg et al. 1996

Table 2 Products formed during the conversion of chlorinated propanes by *M. trichosporium* OB3b

Substrate	Substrate converted (μM)	Products	Product concentration (μM)	Product yield (% of substrate converted)
1-Chloropropane	246	1-Chloro-2-propanol	9	4
		Chloride	164	67
1,2-Dichloropropane	242	1-Chloro-2-propanol	26	11
		2-Chloro-1-propanol	53	22
		2,3-Dichloropropanol	138	57
		Chloride	100	21
1,3-Dichloropropane	243	Chloride	338	70
1,2,3-Trichloropropane	219	2-Chloro-1-propanol	145	66
		1,3-Dichloro-2-propanol	7	3
		2,3-Dichloro-1-propanol	24	11
		Chloride	410	62

cyclopropane-treated cells, 67% of the added 3-chloro-1-propanol was left and 17% of the organic chlorine was released as chloride. These results indicate that 1-chloropropane was mainly converted to 3-chloro-1-propanol, which was rapidly further transformed and dehalogenated by a route that slowed down in the presence of cyclopropane, suggesting that the methanol dehydrogenase was involved in the conversion of 3-chloro-1-propanol.

Accumulation of 2,3-dichloro-1-propanol, 1-chloro-2-propanol and 2-chloro-1-propanol was observed during transformation of 1,2-dichloropropane by resting cells of *M. trichosporium* OB3b. The conversion of 1,2-dichloropropane in a batch incubation was followed in time by GC analysis to identify early intermediates (Fig. 2). The first products of 1,2-dichloropropane conversion were 2,3-dichloro-1-propanol and chloroacetone. As chloroacetone was degraded, a concomitant increase of 1-chloro-2-propanol was observed, indicating a reduction. In a separate batch experiment cells (0.7 mg (dry weight)/ml) were incubated with 0.6 mM 2,3-dichloro-1-propanol. After 65 h 420 μM 2,3-dichloro-1-propanol was left and 150 μM 2-chloro-1-propanol and 200 μM chloride were produced. No degradation of 2,3-dichloro-1-propanol was observed in a control incubation with heat-killed cells. These results indicate that reductive hydrogenolysis of 2,3-dichloro-1-propanol did indeed occur.

After conversion of 1,3-dichloropropane by *M. trichosporium* OB3b, no propane derivatives could be detected. At the end of the experiment, most of the chlorine added as 1,3-dichloropropane was present as inorganic chloride. Considering the high inactivation constant found for this compound, toxic products must be formed during conversion.

Low amounts of 2,3-dichloro-1-propanol and 1,3-dichloro-2-propanol were formed during conversion of

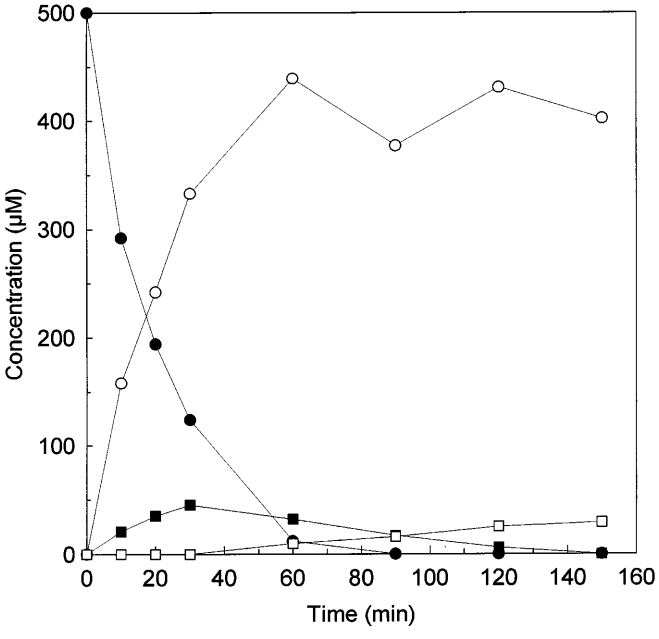

Fig. 2 Conversion of 1,2-dichloropropane (0.5 mM) by *M. trichosporium* OB3b. The cell concentration was 0.82 mg/ml. (●) 1,2-Dichloropropane, (○) 2,3-dichloro-1-propanol, (■) chloroacetone, (□) 1-chloro-2-propanol

1,2,3-trichloropropane, and 62% of the organic chlorine was liberated as inorganic chloride. It was found that 2-chloro-1-propanol was the main product of 1,2,3-trichloropropane conversion, again indicating reductive dechlorination.

Reaction pathway of 1,2,3-trichloropropane conversion

The soluble methane monooxygenase of *M. trichosporium* OB3b inserts oxygen into C-H bonds. With 1,2,3-trichloropropane this would lead to the formation of 2,3-dichloropropionaldehyde or 1,3-dichloroacetone, depending on whether oxygen insertion takes place on the C-1 or C-2 carbon (Fig. 3). The observation that monochlorinated and dichlorinated propanols were formed during conversion of 1,2-dichloropropane and 1,2,3-trichloropropane, respectively, implies additional reaction pathways.

The formation of 1,3-dichloro-2-propanol and 2,3-dichloro-1-propanol from 1,2,3-trichloropropane could occur via reduction of 1,3-dichloroacetone or 2,3-dichloropropionaldehyde respectively (Fig. 3, routes a and b). Reduction of an aldehyde function by *M. trichosporium* OB3b has been demonstrated for trichloroacetaldehyde (chloral hydrate), which was partly reduced to trichloroethanol (Oldenhuis et al. 1989; Newman and Wackett 1991). Other explanations for the formation of dichloropropanols from 1,2,3-trichloropropane could be insertion of oxygen into the C-Cl bond (Fig. 3, routes c and d), or substitutive displacement of the halogen, as has been proposed for 1,2-dichloroethane, chloroacetic acid and chloroacetamide (Riebeth et al. 1992; Castro et al. 1996).

Upon reduction of the aldehyde or ketone, two new hydrogen atoms would be introduced (Fig. 3, routes a and b), which must be derived from formate via hydride transfer with NADH or from the solvent via protons and electron transfer. Only one hydrogen would be introduced by direct substitution (routes c and d). In both cases, one hydrogen is on the hydroxyl group and can exchange with the solvent. The difference between these routes is that an additional carbon-bound deuterium should be introduced, either with deuterated formate or

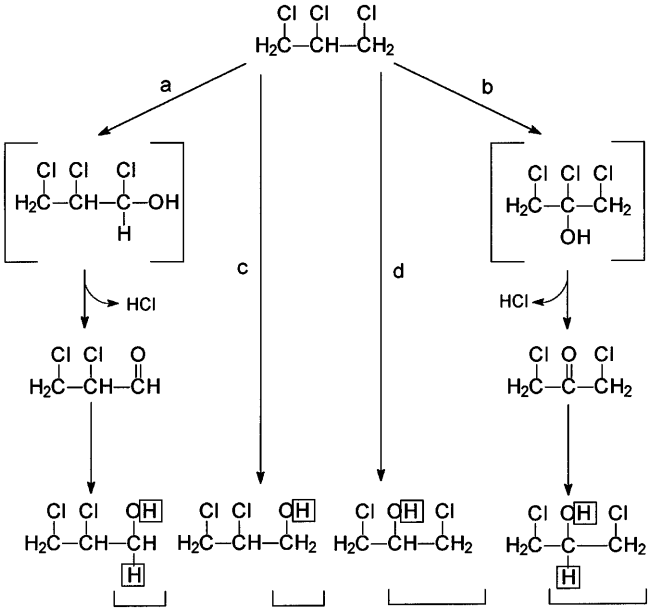

Fig. 3 Possible conversion routes of 1,2,3-trichloropropane by *M. trichosporium* OB3b. The relevant fragments (for 2,3-dichloro-1-propanol: *m/z* 31, for 1,3-dichloro-2-propanol: *m/z* 79, 81) for differentiation between routes *a*, *b* and *c*, *d* are underlined. The formation of dichlorinated propanols could occur via reduction of the aldehyde or ketone (routes *a* and *b*) or via direct insertion of oxygen into the carbon-halogen bond of 1,2,3-trichloropropane (routes *c* and *d*). The hydrogens introduced are indicated in boxes

with deuterium oxide, if reduction is involved in the formation of the alcohol. To distinguish between these possibilities, we conducted experiments in which 1,2,3-trichloropropane was converted in the presence or absence of deuterium oxide and deuterated sodium formate, and the number of introduced hydrogens was determined by GC-MS analysis of the products (Table 3). The major fragments found for commercial 2,3-dichloro-1-propanol were: *m/z* 92, 94 (C_3H_5ClO); *m/z* 62, 64 (C_2H_3Cl); *m/z* 31 (CH_3O); and for 1,3-dichloro-2-propanol: *m/z* 79, 81 (C_2H_4ClO); *m/z* 49, 51 (CH_2Cl). The characteristic 3:1 ratio was found for the fragments *m/z* 79, 81 of 1,3-dichloro-2-propanol, because of the natural abundances of ^{35}Cl and ^{37}Cl. The relevant fragments for differentiation between the two

Table 3 Incorporation ^2H during conversion of 1,2,3-trichloropropane (TCP) by whole cells of *M. trichosporium* OB3b. Cells were incubated in buffers containing ^2H_2O as solvent or sodium [^2H] formate (^2HCOONa). The dichloropropanols formed were analysed for deuterium content. The buffers used for the incubations were as described in Materials and methods

Substrate	Buffer	Relative abundances of products							
		2,3-Dichloro-1-propanol			1,3-Dichloro-2-propanol				
		m/z 31	*m/z* 32	*m/z* 33	*m/z* 79	*m/z* 80	*m/z* 81	*m/z* 82	*m/z* 83
Reference	H_2O[a]	100	0	0	100	3	32	0.6	0.03
	^2H_2O[a]	100	114	0	100	178	36	58	1.5
TCP	2HCOONa[b]	100	0	0	100	0	32	0	0
	^2H_2O[c]	100	443	84	100	179	160	100	42

[a] 2,3-Dichloro-1-propanol or 1,3-dichloro-2-propanol dissolved in H_2O or ^2H_2O without cells
[b] Deuterated sodium formate was used as a source of reducing equivalents
[c] Freeze-dried buffer dissolved in deuterium oxide

reaction pathways were m/z 31 (CH_3O) for 2,3-dichloro-1-propanol and m/z 79, 81 (C_2H_4ClO) for 1,3-dichloro-2-propanol. Upon incubation of 2,3-dichloro-1-propanol in deuterium oxide without cells, the relevant fragments were m/z 31 (CH_3O); m/z 32 (C^2HH_2O); and for 1,3-dichloro-2-propanol: m/z 79 ($C_2H_4{}^{35}ClO$); m/z 80 ($C_2{}^2HH_3{}^{35}ClO$); m/z 81 ($C_2H_4{}^{37}ClO$); m/z 82 ($C_2{}^2HH_3{}^{37}ClO$). The shift of one mass unit is explained by chemical exchange of hydrogen and deuterium on the hydroxyl group of the dichloropropanols.

No deuterium was incorporated during conversion of 1,2,3-trichloropropane by *M. trichosporium* OB3b in the presence of deuterated sodium formate, since the mass spectra were similar to those for dichloropropanols dissolved in H_2O. If an NAD-dependent dehydrogenase has the opposite stereospecificity to that of the NAD-dependent formate dehydrogenase, the hydrogen of NAD^2HH will be transferred to the product instead of the deuterium. However, owing to recycling of the NAD/NADH pool via the formate dehydrogenase, both positions on the carbon-4 of NAD will be occupied by a deuterium, which will end up in the product after repeated turnover. During this experiment, 150 μmol 1,2,3-trichloropropane was converted by 320 mg cells. Assuming a cell volume of 1.7 ml/g cells (dry weight) and an intracellular NAD/NADH concentration of 2 mM (Snoep et al. 1994), it can be calculated that the NAD/NADH pool was recycled 150 times during 1,2,3-trichloropropane conversion.

Incorporation of deuterium did occur during conversion of 1,2,3-trichloropropane in the presence of deuterium oxide. The relative abundances of mass fragments m/z 32 and 33 for 2,3-dichloro-1-propanol and mass fragments m/z 81, 82 and 83 for 1,3-dichloro-2-propanol were higher than in the control, which contained deuterium oxide but no cells. Furthermore, the two mass fragments m/z 33 (C^2H_2HO) for 2,3-dichloro-1-propanol and m/z 83 ($C_2{}^2H_2H_2{}^{37}ClO$) for 1,3-dichloro-2-propanol were only observed in the presence of cells. Thus two deuteriums were incorporated during oxidation of 1,2,3-trichloropropane.

The formation of 1,3-dichloro-2-propanol via reduction of 1,3-dichloroacetone was tested separately by incubating 500 μM 1,3-dichloroacetone at 30 °C for 2 h. A low amount of 1,3-dichloro-2-propanol (46 μM) was detected. No formation of 1,3-dichloro-2-propanol was observed in a control incubation with heat-killed cells. Dichloropropionaldehyde was not tested, because this compound was not available.

The above results indicate that the dichloropropanols were produced during oxidation of 1,2,3-trichloropropane via reduction of 2,3-dichloropropionaldehyde and 1,3-dichloroacetone (Fig. 3, routes a and b).

Discussion

The results of this work show that cells of *M. trichosporium* OB3b expressing the soluble form of the methane monooxygenase can cometabolically transform chlorinated propanes. The first-order rate constants decreased in the order of 1-chloropropane, 1,3-dichloropropane, 1,2-dichloropropane and 1,2,3-trichloropropane. The conversion rates thus decreased with an increasing number of chlorine substituents. The first-order rate constant of 1,2,3-trichloropropane was similar to that previously found for 1,1,1-trichloroethane (0.1 ml min^{-1} mg $cells^{-1}$). The highest rate constant was observed for 1-chloropropane, which was similar to that for 1,2-dichloroethane and 1,1-dichloroethylene (1.0 ml min^{-1} mg $cells^{-1}$) (Oldenhuis et al. 1991). However, compared to an important environmental pollutant such as trichloroethene, chlorinated propanes are poor substrates.

All chlorinated propanes tested exhibited product toxicity, resulting in a finite transformation capacity (Alvarez-Cohen and McCarty 1991). The transformation capacities varied from 1.47 μmol/mg for 1-chloropropane and 1,2-dichloropropane to 0.62 μmol/mg for 1,3-dichloropropane. This is low compared to the values found for trichloroethene (2.0 μmol/mg) or 1,2-dichloroethane (10 μmol/mg) under batch incubation conditions (Chang and Alvarez-Cohen 1996). The transformation capacities of chlorinated methanes, ethanes and ethenes were proposed to be inversely proportional to their chlorine content (Chang and Alvarez-Cohen 1996). Such a clear relation was not found for the chlorinated propanes.

Product analysis showed that oxygen insertion was preferred on the non-substituted carbon since 3-chloro-1-propanol and 2,3-dichoro-1-propanol accumulated during conversion of 1-chloropropane and 1,2-dichloropropane respectively. The results suggest that the lack of a terminally unsubstituted carbon atom, as for 1,3-dichloropropane and 1,2,3-trichloropropane, increases the toxicity of these compounds. Insertion of oxygen on a chlorine-substituted carbon would yield carbonyl compounds which are probably more reactive than chlorinated propanols (Henschler 1985). Furthermore, hydrogen abstraction, which is assumed to be the first step in substrate oxidation, leads to a chloroalkyl radical, which may be extremely reactive towards nucleophilic groups in the protein. Experiments with trichloro-[^{14}C]ethene have shown that its toxicity is caused by a nonspecific reaction of conversion products with cell components, including the soluble methane monooxygenase (Oldenhuis et al. 1991; Fox et al. 1990).

The main product of 1-chloropropane conversion, 3-chloro-1-propanol, was only detected with cells in which alcohol dehydrogenase was inhibited by cyclopropane. The relative amounts of the products formed were somewhat different from those found by Shimoda et al. (1993). They used cyclopropanol as a selective inhibitor for methanol dehydrogenase and found accumulation, in increasing concentrations, of 3-chloro-1-propanol, 1-propanol and 1-chloro-2-propanol. In our experiments with cyclopropane-treated cells, 3-chloro-1-propanol was the major degradation product, and we detected only low amounts of 1-chloro-2-propanol and no

1-propanol. The difference may be due to variations in cell density, monooxygenase activity, or incubation times.

The amounts of chloride released during the conversion of 1-chloropropane, 1,3-dichloropropane and 1,2,3-trichloropropane were similar. Since 1,2-dichloropropane was mainly converted to 2,3-dichloro-1-propanol, less chloride was released. In the timescale of the experiment (200 min) 2,3-dichloro-1-propanol was almost not converted, although conversion to 2-chloro-1-propanol is possible, probably via reductive hydrogenolysis. Such a reaction has been proposed for the conversion of chloroethylene oxide to ethylene oxide by *M. trichosporium* OB3b (Castro et al. 1992). The formation of 2-chloro-1-propanol during transformation of 1,2,3-trichloropropane also points to reductive dechlorination occurring with *M. trichosporium* OB3b. In this case 2,3-dichloropropionaldehyde should be an intermediate that is converted to 2-chloro-1-propanol by a combination of carbonyl reduction and reductive hydrogenolysis. These reductive dechlorination reactions occurred in batch cultures in which excess oxygen was present and mass transfer of oxygen was rapid, indicating that reductive dechlorination can occur under aerobic conditions. Whether the methane monooxygenase is involved in this conversion or biomolecules such as a cytochrome or a reduced coenzyme are responsible is not yet known. Reductive dechlorination by mammalian cytochrome *P*-450 has been well established (Henschler 1985).

Another type of reaction that influenced the nature of the products formed from chloropropanes was reduction of carbonyl groups. This reaction explains the formation of 1-chloro-2-propanol from 1,2-dichloropropane, and of both 2-chloro-1-propanol and 1-chloro-2-propanol during conversion of 1,2,3-trichloropropane. For the latter compound it was found that 2,3-dichloro-1-propanol and 1,3-dichloro-2-propanol were formed via reduction of the aldehyde or ketone and that the hydrogens introduced were derived from water. This was further confirmed by using 1,3-dichloroacetone as the substrate, which yielded 1,3-dichloro-2-propanol as the product, while in a heat-killed control no 1,3-dichloro-2-propanol was found. So, 1,2,3-trichloropropane was probably mainly converted to 1,3-dichloroacetone and to 2,3-dichloropropionaldehyde. A small fraction of these products was reduced to the corresponding alcohols. The reduction is a minor conversion since only low amounts of dichloropropanols were found and their reduction was very slow. An initial insertion of oxygen into the C-Cl bond as a major reaction pathway is not likely, because larger amounts of the dichloropropanols would accumulate and the observed incorporation of hydrogen from water into carbon-bound hydrogen of the product would not occur. This is in contrast with conversion of 1,2-dichloroethane for which it was proposed that oxygen was mainly inserted into the carbon-chlorine bond (Riebeth et al. 1992).

The results of this work show that *M. trichosporium* OB3b cometabolically converts chlorinated propanes. Compared to other important environmental pollutants, the transformation capacities were low. Bacterial growth has been observed on 1-chloropropane and 1,3-dichloropropane, which, of course, is preferable to cometabolic conversion. However, for recalcitrant compounds such as 1,2-dichloropropane and 1,2,3-trichloropropane, cometabolic conversion by *M. trichosporium* OB3b may be an alternative. The range of products formed is determined by at least four reactions: carbon hydroxylation, alcohol oxidation, carbonyl reduction and reductive dechlorination.

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